EFFECT OF LIPID INTAKE ON DIRECT HYPERBILIRUBINEMIA IN LATE PRETERM AND TERM INFANTS WITH GASTROINTESTINAL SURGICAL PROBLEMS

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I. PURPOSE OF THE STUDY AND BACKGROUND

The purpose of the study is to establish a cause and effect relationship between lipid intake and the incidence of direct hyperbilirubinemia (cholestasis) in late preterm and term infants with major gastro-intestinal (GI) surgical disorders such as gastroschisis omphalocele, volvulus, TE fistula, duodenal atresia, jejunal atresia, ileal atresia, hirschsprung's disease, anorectal malformation, intestinal obstruction and GI perforations requiring surgery. Infants with major GI surgical disorders as mentioned above will be randomized to receive 1gm/kg/day or 2gm/kg/day of 20% intralipids within the first 72 hours after birth. The study is not open to all surgical infants because infants with other surgical problems can be fed enterally within few days after surgery and usually do not require TPN for prolong periods (>2 weeks). Also infants with major GI surgical disorders have similar clinical course, undergo similar type of operative procedures, and have similar complications thus eliminating potential confounding factors. The primary outcome measure is the incidence rate of parenteral nutrition associated cholestasis (PNAC). PNAC will be defined as serum direct bilirubin ≥ 2mg/dl in the absence of other known causes of direct hyperbilirubinemia as described under exclusion criteria.

II. BACKGROUND AND JUSTIFICATION:

Since its introduction in the late 1960s, total parenteral nutrition (TPN) has established itself as an effective mode for parenteral hyperalimentation in patients with acute or chronic intestinal failure. Out of the many complications of parenteral nutrition, the most common is the cholestasis often referred to as Parenteral Nutrition Associated Cholestasis (PNAC). PNAC is defined clinically using

direct bilirubin as an index measure in the absence of other known causes of direct hyperbilirubinemia (see exclusion criteria). The reported incidence of PNAC in neonates is as high as 84%. [1, 2]

PNAC was first described by Peden and colleagues [3] and has long been recognized as a major cause of morbidity and mortality in neonates and infants. [4, 5] PNAC can be associated with liver dysfunction, cirrhosis, and hepatic carcinoma. The proposed mechanisms that could explain cholestasis caused by the total parenteral nutrition are multifactorial. The major mechanisms include hemodynamic modifications within the biliary system caused by parenteral administration of nutrients, bile stasis resulting from decreased stimulation of gastrointestinal hormones in the absence of any enteral intake, direct toxic effects of TPN components, premature biliary system, and bacterial overgrowth leading to overproduction of deconjugated bile acids. Very few observational studies done earlier have looked at the association of PNAC with various nutritional components of TPN. The findings of these studies indicate that PNAC could be associated with increased duration of TPN [6, 7] with almost all infants developing PNAC by 6 weeks on TPN [8,9,10], higher intake of amino acids [11], higher intake of dextrose, intake of trace elements like aluminium [12], copper and manganese [13], and cumulative amount of intravenous lipid intake[14]. Several preventive trials have been conducted in neonates involving the use of different amounts of protein intake or the use of choleretic medications in order to prevent cholestasis. Vileisis and colleagues [11] did not find any difference in the incidence of PNAC when they compared amino acids intake of 2.3gm/kg/day vs. 3.6 gm/kg/day. Their findings were reciprocated by Clark and colleagues [15] who found no difference in the incidence of PNAC when comparing amino acids supplementation of 2.5 vs. 3.5gm/kg/day. Similarly, the use of both cholecystokinin (CCK) [16], and ursodeoxycholic acid [17] failed to prevent the development of PNAC. Majority of these preventive trials performed in neonates defined PNAC as direct bilirubin ≥2mg/dL after two weeks on PN [9, 11, 21]. Because these preventive trials failed to identify any

preventive therapy, there is a need to identify alternative ways to prevent PNAC in high-risk infants.

Recent literature including our finding from an observational study suggests that cumulative lipid intake may be associated with PNAC in high-risk infants with GI surgical disorders. The side effects of intravenous lipid infusions on the hepato-billiary system was first suggested in animal studies in 1993 when La Scala et al. described that the addition of lipids increases TPN associated cholestasis in rats [18]. Haynes et al. in 2002 published a series of hepatic pathology and described dose dependent hepatic changes with prolonged administration of lipid in premature baboons which included steatosis, cholestasis, and proliferation of bile ducts [19]. Since then few observational studies in neonates have been performed and demonstrated that lipid intake is associated with PNAC. [7, 11] However, a cause and effect relationship between lipid intake and PNAC has not been established using a well-designed randomized clinical trial. We hypothesize that increased cumulative amount of lipid intake causes PNAC in late preterm and term neonates with major GI surgical disorders. Our objective is to evaluate the effect of cumulative amount of lipid intake on the incidence of PNAC in this vulnerable population.

III. CHARACTERISTICS OF THE RESEARCH POPULATION

1. **Number of Subjects:** This is a prospective randomized clinical trial involving 40 neonates with major GI surgical disorders requiring surgery.

2. **Gender, Age, Race, and Ethnicity:** The University of Rochester Medical Center (URMC) is a tertiary care center and consists of a 52 bed Neonatal Intensive Care Unit. Annually, ~25 infants with major GI surgical disorders are admitted to the URMC. The NICU population is approximately 52%

male with a racial distribution of 64% White, 32% African-American, 2% Asian, and 2% other. The ethnic distribution is 75% non-Hispanic. The gender, race, and ethnicity distribution of this prospective study is expected to follow the distribution of the NICU population.

3. **Inclusion Criteria**: All neonates ≥ 34 weeks gestational age with major GI surgical disorders (gastroschisis, omphalocele, volvulus, trachea-esophageal fistula, duodenal atresia, jejunal atresia, ileal atresia, hirschsprung's disease, anorectal malformation, intestinal obstruction, and GI perforations) requiring surgery admitted to our NICU within first 72 hours will be eligible for this study.

4. **Exclusion Criteria:** Neonates with the following condition will be excluded: 1) If does not need TPN by 72 hours; 2) Direct hyperbilirubinemia within the first 72 hours after birth; 3) TORCH infections (Toxoplasmosis, CMV, Herpes, Rubella, HIV, etc); 4) Biliary tract disorders leading to direct hyperbilirubinemia; 5) Known metabolic disorders that may be associated with direct hyperbilirubinemia- such as Galactosemia, α-1 antitrypsin deficiency, etc.

IV. METHODS AND PROCEDURES

a) Study Design: This is a prospective randomized controlled trial s planned in order to evaluate the incidence rate of PNAC between 1gm/kg/day of 20% intralipid and 2 gm/kg/day of 20% intralipid in high-risk neonates ≥ 34 weeks gestational age (GA). PNAC will be defined as serum direct bilirubin ≥ 2mg/dl in the absence of other known causes of direct hyperbilirubinemia as described in the exclusion criteria.

b) Screening: All neonates ≥ 34 weeks GA and admitted to the NICU at URMC with major GI surgical disorders will be screened for study criteria within 72 hours after birth. Infants will be

identified by a designated investigator with regularly scheduled screening of admissions, GA, postnatal days and admission diagnosis. Screening will take place on an ongoing basis and not less than 3 times per week. The screening log will include GA, postnatal days, and type of GI surgical disorders, and whether or not the infant was enrolled in the study. If not enrolled, the reason will be recorded. The number and demographic characteristics of eligible subjects not included will be collected to assess the representation of the sample of subjects included.

c) **Enrollment and Consent**: Families will be approached by one of the investigator as soon as possible after eligibility is ascertained to allow sufficient time for the family to ask questions about the study. Parental consent may be obtained any time during the first 72 hours after birth. Consent will be documented on an IRB approved consent form. Each subject will be assigned a unique study identification number by the investigator after consent is obtained.

d) **Pre-randomization**: Once the infant is enrolled in the study, one of the investigators will notify the Research Pharmacist (RP) by telephone call during working hours (8AM-5PM) for registration and randomization. Information on weight of the infant will be provided to the RP for accurate dosing of intravenous lipid on each day.

e) **Registration**: A log sheet of subjects registered with their names, study ID numbers, medical record number, dates of birth, and dates of randomization will be kept by the RP

f) **Randomization** will be to two arms: 1g/kg/day intravenous lipid or 2 g/kg/day for 6 weeks or until develops direct bilirubin ≥ 2mg/dl (in the absence of sepsis) or reaches enteral feeds and no longer requires parenteral nutrition. A block randomization with 8 subjects in each block will be performed. Study group assignments will be made by the RP with the use of a randomization scheme provided by the biostatistician. The assignment will be 1:1 ratio.

g) **Route, Frequency, and Duration**: The lipids will be given intravenously every day as per the standard of care for 6 weeks or until reach one of the study end points (reach enteral feeds and no longer require parenteral nutrition or has direct bilirubin ≥ 2 mg/dl in the absence of sepsis).All enrolled infants including those randomized to 2 gm/kg/day intravenous lipid will be started at 1gm/kg/day of intravenous lipids as per the standard of care on day 2 after birth. The procedures for titrating subjects randomized to the higher dose will be consistent with standard procedures. The daily intravenous lipid intake will be increased to 2 gm/kg/day in those randomized to 2gm/kg/day. The amount of proteins and glucose will be ordered as per the unit policy and at the discretion of the attending neonatologist.

h) **Dose Adjustments**: Daily assessment of the weight will be performed by the investigator and will be reported to the RP for appropriate adjustment to the dosing of the intravenous lipid. Each day, the RP will inform the clinical care team of volume of intravenous lipid to be infused over the day. The clinical care team will complete the request in the E-record using the information provided by the investigator or the RP.

i) Subjects will receive glucose, protein, vitamins, and trace elements at the discretion of the attending neonatologist as per the standard of care. The unit policy regarding nutrition is to gradually increase protein intake to 3 to 4 gm/kg/day and intravenous glucose intake as tolerated to provide a caloric intake of 90 – 110 kcal/kg/day. Assuming the average intake of 120ml/kg/day of TPN, the predicted amount of caloric intake from the non-fat portion of the TPN would be ~ 90 kcal/kg/day (assuming protein intake of 3 to 4 gm/kg/day and an average dextrose concentration of 15% via the central line). The clinical team will assume an average lipid intake of 1.5gm/kg/day for each enrolled infant during the period of the study which is equivalent to a caloric intake of 13.5 ± 4.5 kcal/kg/day from lipid intake. Thus the total caloric

intake for the infants in the study would be 90 + 13.5 ± 4.5 Kcal/kg/day or 103.5 ± 4.5 Kcal/kg/day. If the enrolled subject does not gain weight despite reaching allowable maximum protein supplementation, there will be room for increasing caloric intake by increasing the amount of glucose intake (either by increasing dextrose concentration or increasing total fluid intake). Increasing glucose intake for weight gain is the standard of care in our NICU while limiting lipid intake in neonates at risk for cholestasis.

j) In case of hypertriglyceridemia, the unit protocol of intravenous lipid intake will be used and the RP will be notified. According to the unit policy, the Intravenous lipid intake will be halved when the serum triglycerides level is between 250 to 350 mg/dl and would be stopped when the serum triglycerides is >350 mg/dl. Once the hypertriglyceridemia has resolved, the assigned intravenous lipid intake will be resumed as per the randomization arm. The discontinuation of trace elements and the administration of choleretic medications will be done as per the unit policy. According to the unit policy, the use of trace elements will be discontinued if direct bilirubin is ≥ 2 mg/dl. Similarly, the unit policy is to add choleretic medications when direct bilirubin level is ≥ 2 mg/dl. The care team will be requested to adhere to these unit policies during the conduct of the study.

k) **Primary Outcome Measures:** PNAC will be the primary outcome measure and will be defined as direct bilirubin ≥ 2mg/dl developing within one week of the completion of 6 week randomization period in the absence of other known causes (discussed earlier). Total serum bilirubin and direct bilirubin concentration will be measured once weekly as part of the standard of care when on parenteral nutrition. The direct bilirubin is usually measured until the resolution of direct hyperbilirubinemia as part of the standard of care. If parents have consented, we will collect blood that is leftover from blood draws that are done as part of standard of care.

The blood samples will be stored in -80 degree freezer in the PI's laboratory within the Department of Neonatology for possible future research on intravenous nutrition and its effects on the liver such as identifying a more sensitive biochemical marker than direct bilirubin concentration of liver injury. Samples will be stored with a study number only and will only be linked to the data collected for the purposes of this study. Only study team members will have access to the stored blood and will be able to use the samples. Future research on stored blood after completion of this study will only be conducted by the PI on IRB approval.

l) **Secondary Outcome measures:** Secondary outcome measures will include peak direct bilirubin concentrations during hospital stay, use of choleretic medications and use of Omegavan during the hospital stay and percentage weight gain during the 6 week study period.

m) **Data Safety Monitoring:** There are several reasons why independent data safety monitoring and stopping rules were not considered for this study as described below:

1) In this study, the outcome measure, direct hyperbilirubinemia >2.0mg/dL, is the potential side effect of interest secondary to intravenous lipid intake. As per the standard of care, the intravenous lipid intake of 1-2 gm/kg/day is routinely used in the presence of direct hyperbilirubinemia of 2 mg/dL to provide essential calories and prevent essential fatty acid deficiency. Usually, choleretic medications such as cholecystokinin are used to resolve PNAC at this point.
2) Our hypothesis is that the development of PNAC is causally related to the cumulative lipid intake, however, protein and glucose intake may modify the association between lipid intake and PNAC. In addition, sepsis and prematurity are confounding risk factors. The sample size calculated (n=40) to evaluate the outcome measure (or the adverse event) accounts for the variable duration of the TPN (cumulative lipid intake) and presence of confounding risk factors. Therefore, meaningful statistical analysis is not possible with an interim analysis of less than 40 subjects.
3) As suggested by our observational study, a mean cumulative dose of 55gm/kg (SD 15) of intravenous lipid is required before the development of PNAC in late preterm and term

infants. Thus, it is expected that an infant on 1gm/kg/day of intravenous lipid arm will take ~ 55 days to develop PNAC. Therefore, considering the fact that TPN intake will often be less than 42 days among study subjects, not all infants in either group are expected to develop PNAC. PNAC, the study outcome, as defined for the purpose of the study, is the earliest when any medications are used to treat PNAC as per the standard of care. Severe PNAC is defined when direct bilirubin concentration is > 10 mg/dL and is very rare. The randomization of intravenous lipid intake will be stopped long before severe PNAC may be seen among enrolled subjects.

4) Performing interim analyses will require more stringent significance level which will increase the sample size required to perform the study. This is a pilot study performed as part of fellow's project for which 40 subjects are required for meaningful analysis of outcome (or adverse event)
5) This study has been designed as a block randomization with each block having 8 subjects with 1:1 assignment, so even if all enrolled infants were to develop PNAC, it will suggest that there is no difference between the two groups and there is no causal association between intravenous lipid intake and cholestasis.

Because of reasons mentioned even if the first 20 subjects (50%) hit the direct bilirubin endpoint, interim analysis is unlikely to allow meaningful analysis of the causation between lipid intake and the outcome measure (or adverse event) as provided in the protocol. In other words, it is unlikely to reach adjusted statistical significance required for interim analyses. Thus, since the outcome measure and the adverse event are the same and the sample size required to evaluate the association (or the adverse event) controlling for other factors is 40, no interim analysis and stopping rules were considered for this pilot study. For trial management, the investigation team will track enrollment and protocol compliance and review every 3 months.

V. <u>DATA COLLECTION</u>: The baseline data on demographics and clinical factors (antenatal and postnatal) before randomization on each subject will be collected. Similarly, data on clinical factors will be collected during the 6 week study period or until the subject is receiving intravenous lipid as per

randomization or until the subject develops PNAC (direct bilirubin ≥ 2 mg/dl in the absence of concurrent infection).

The information on TPN components, specifically amount of lipid, protein, and glucose intake on each day will be prospectively collected using pharmacy and TPN records. Information on cumulative amount of lipid intake, cumulative amount of protein intake, and cumulative amount of glucose intake will be determined for all enrolled subjects. The additional information that will be collected is duration (days) of TPN, use of trace elements, duration (days) of NPO, type of enteral feeds (maternal breast milk vs. Formula) duration (days) of antibiotic use, onset and duration of choleretic medications, sepsis defined as blood culture positivity or clinical signs and symptoms of sepsis with the use of antibiotics for > 3 days, day of life when the infant reaches an enteral intake of 120 ml/kg/day, duration (days) of mechanical ventilation, duration of CPAP, duration of oxygen supplementation, and amount of erythrocyte transfusions during the study period. However, use of choleretic medications and discontinuation of trace elements from the TPN is not expected during the study period based on the unit policy as delineated earlier.

VI. <u>SAMPLE SIZE CALCULATION</u>: Assuming a 4-week median follow-up time and an incidence rate of 40% for 6 weeks in control group, a two-sided log rank test with an overall sample size of 40 subjects (20 per group) achieves 80.0% power at a 0.050 significance level to detect a hazard ratio of 3.6.

Power	Incidence Rate	N Control	N Study	Haz Ratio (HR)
0.8	40%	20	20	3.6
0.8	50%	20	20	3.26
0.8	60%	20	20	3.02

Kaplan-Meier (KM) estimates of survival function will be provided and compared between groups. Log-rank test will also be used to compare time to endpoint. Cox proportional hazards model will be fitted after controlling for covariates which are found to be imbalanced in preliminary analysis. Estimate of hazard ratio and associated confidence interval will be obtained.

VII. DATA ANALYSES AND MONITORING:

a) Randomization: Upon successful completion of necessary screening assessments and being confirmed to be eligible by the investigator, subjects will be randomized in 1:1 ratio to two groups: 1) 1 g/kg/day intravenous lipid and 2) 2 g/kg/day intravenous lipid. Each subject will be assigned a unique sequential subject number for identification throughout the entire course of the study. The subject number will not be reused for any other participant in the study. Subjects who are discontinued or withdrawn from the study prior to randomization will be replaced. In addition, subjects who are identified to have direct hyperbilirubinemia from causes other than parenteral nutrition or did not require parenteral nutrition for more than 2 weeks will be considered screen failure and will also be replaced. We anticipate a total number of 52 subjects to participate in the study to complete the study on 40 subjects. During the study, the treatment assignments will be available only to the RP.

c) Samples for Analysis: The "Intent-to-Treat" sample includes all subjects who are randomized into the study. Subjects will be grouped according to the treatment to which they are randomized.

d) Statistical Analyses:

General statistical Considerations:

All statistical analyses will be conducted using Version 10 of the SAS System for Windows (Copyright © 2002-2003 SAS Institute Inc., Cary, NC, USA.) by a biostatistician. Descriptive statistics will be used to summarize outcomes by treatment groups. For continuous variables, descriptive statistics will include the mean, median, standard deviation, minimum, maximum, number of available observations, and number of missing observations. For discrete variables, descriptive statistics will include frequencies and percentages, number of available observations and numbers of missing observations.

We will determine whether there are important baseline differences between the two groups despite randomization with respect to participant characteristics (e.g., gender, race/ethnicity, GA at birth, SGA[$<10^{th}$ percentile]). If any important differences are found, the primary analyses will be repeated after statistically adjusting for these differences. If distributional assumptions associated with a particular statistical procedure are violated, appropriate transformations will be made or non-parametric alternatives will be used (e.g., Wilcoxon rank sum tests in place of two-sample t-tests). Infants enrolled but subsequently found to meet exclusion criteria will be analyzed separately.

The incidence rate of PNAC will be compared between the 2 groups using Kaplan Meier curves. Subjects who reach full enteral feeds before 6 weeks of study period will be censored. Kaplan-Meier (KM) estimates of survival function will be provided and compared between groups. Log-rank test will also be used to compare time to endpoint. Cox proportional hazards model will be fitted after controlling for covariates which are found to be imbalanced in preliminary analysis. Estimate of hazard ratio and associated confidence interval will be obtained.

The potential confounders are sepsis, use of choleretic medications and trace elements. Incidence of nosocomial sepsis has decreased remarkably over the last few years in our unit and with

randomization we do not anticipate significant group differences in the incidence of sepsis. The use of choleretic medications and discontinuation of trace elements should occur after the development of primary outcome as per the unit policy and therefore these factors are unlikely to confound the relationship between the lipid intake and PNAC.

VIII. DATA STORAGE AND CONFIDENTIALITY: We will use unique subject identification number to ensure confidentiality of data collected. The file linking the study identification number with identifiers will be stored in a locked cabinet accessible only to the investigators. Hard copies of data collection forms will be stored in a locked cabinet. All links to subject identifiers will be decoded to protect privacy of subjects. The records will be kept for 5 years after completing the study and publication of the findings.

IX. RISK/BENEFIT ASSESSMENT

This prospective study has been designed to minimize the risks to the patients enrolled. Intravenous lipid intake in the range of 1-2 gm/kg/day is commonly used in high-risk neonates with major GI surgical disorders. PNAC is a common side effect of parenteral nutrition. Although severe cholestasis is defined as direct bilirubin > 10 mg/dl [20] but for the purpose of the study to avoid any risk, we have defined our primary outcome measure as direct bilirubin ≥ 2 mg/dl. Infants with direct bilirubin ≥ 2 mg/dl (reaching primary outcome) will no longer receive randomized lipid intake and the clinical care team will be informed. The lipid intake will then be at the discretion of the attending neonatologist. To protect privacy of subjects, all links to subject identifiers will be decoded and subject data will be stored in locked cabinets.

There is a potential benefit to the study subjects .The benefits to future babies involve more judicious administration of lipids in TPN to avoid cholestasis without depriving them of essential fatty

acids and calories. The findings on the role of cumulative lipid intake for PNAC will be useful for future babies as this intervention may help us to decrease the incidence of PNAC without affecting caloric intake.

X. SUBJECT IDENTIFICATION, RECRUITMENT AND CONSENT

All neonates ≥ 34 weeks GA and admitted to the NICU at URMC with major GI surgical disorders as listed under the inclusion criteria will be screened for study criteria within 72 hours after birth. Infants will be identified by the investigator with regularly scheduled screening of admissions, GA, postnatal days and admission diagnosis. Screening will take place on an ongoing basis and not less than 3 times per week. The screening log will include GA, postnatal days, and type of GI surgical disorders, and whether or not the infant was enrolled in the study. If not enrolled, the reason will be recorded. The number and demographic characteristics of eligible subjects not included will be collected to assess the representation of the sample of subjects included.

Families will be approached by one of the investigator as soon as possible after eligibility is ascertained to allow sufficient time for the family to ask questions about the study. Parental consent may be obtained any time during the first 3 days after birth. Consent will be documented on an IRB approved consent form. Each subject will be assigned a unique study identification number by the investigator after consent is obtained. A log sheet of subjects registered with their names, study ID numbers, medical record number, dates of birth, and dates of randomization will be kept by the RP.

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